Supporting Information

Engineered pH-buffering Nanocomposite for Robust One-Pot Multi-Enzyme Cascade Synthesis of γ-Aminobutyric Acid

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KEYWORDS: Biocatalyst, Enzyme Cascade, Metal Organic Framework, Pharmaceutical Biosynthesis, γ -Aminobutyric Acid

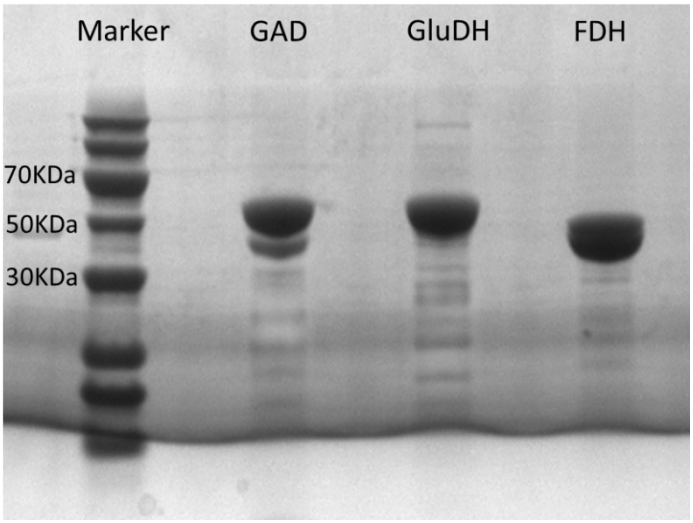

Fig. S1 12% SDS-PAGE protein analysis of purified GAD (55 kDa), GluDH (55 kDa), and FDH (45 kDa).

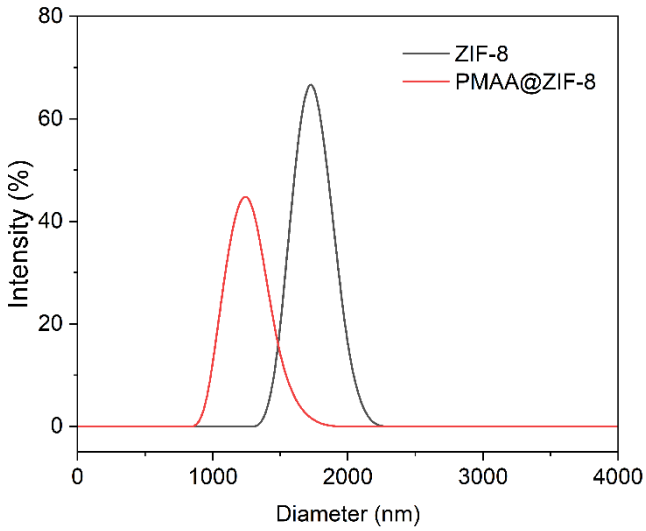

Fig. S2 Dynamic Light Scattering (DLS) Analysis of ZIF-8 and PMAA@ZIF-8.

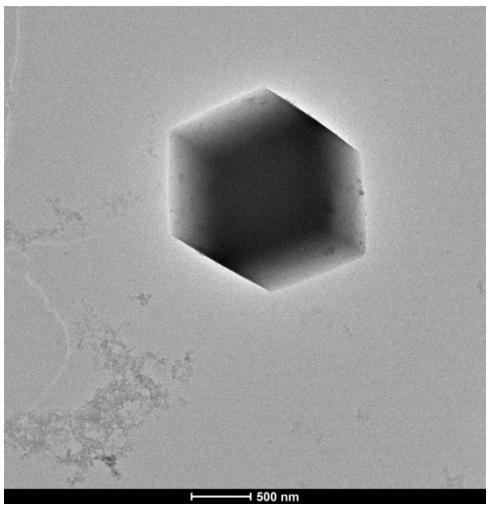

Fig. S3. SEM image of GAD-PMAA@ZIF-8.

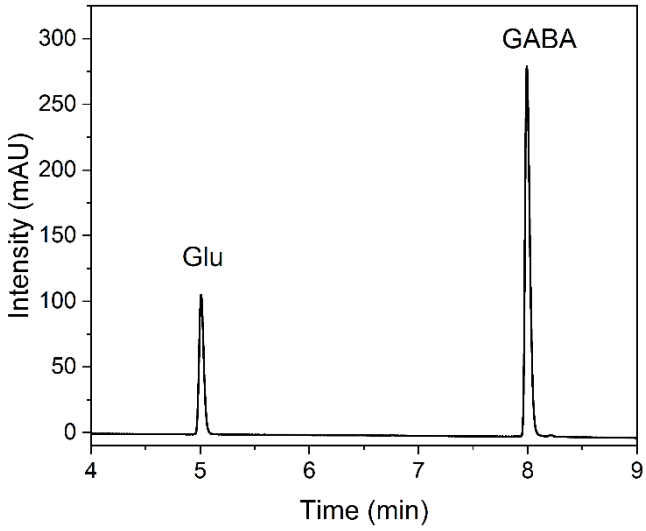

Fig. S4 High-performance liquid chromatography (HPLC) analysis of cascading reactions. The retention times for Glu and GABA are shown at 5 minutes and 8 minutes, respectively.

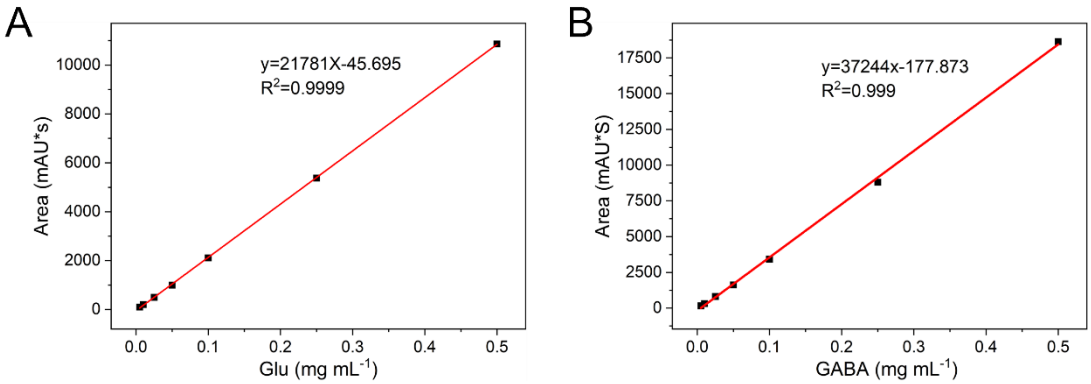

Fig. S5 HPLC calibration curves. (A) HPLC calibration curve for Glu. (B) HPLC calibration curve for GABA.

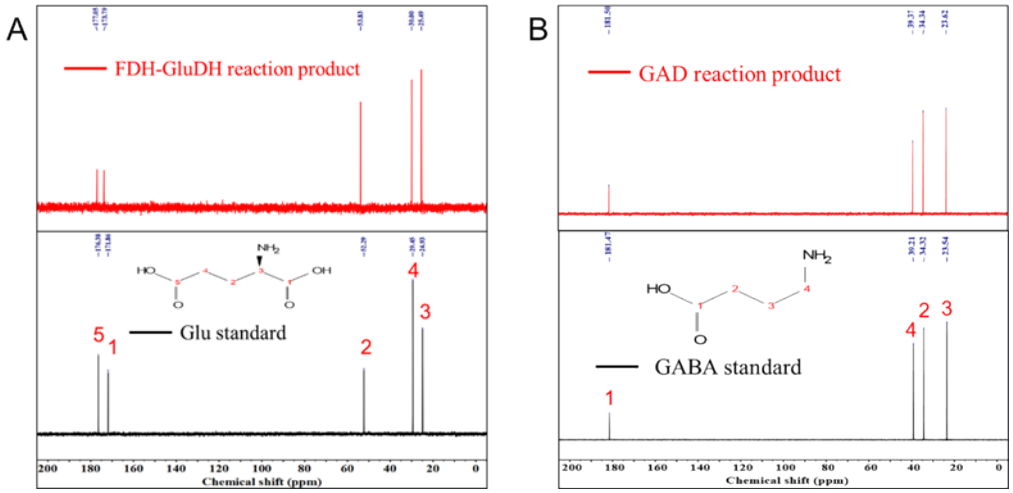

Fig. S6 ^{13}C NMR spectra of the intermediate product glutamate (Glu) and the final product (GABA). (A) Comparison of the 13C spectrum of Glu produced by the cascade reaction of FDH

and GluDH with the standard. (B) Comparison of the ^{13}C spectrum of GABA produced by GAD with the standard.

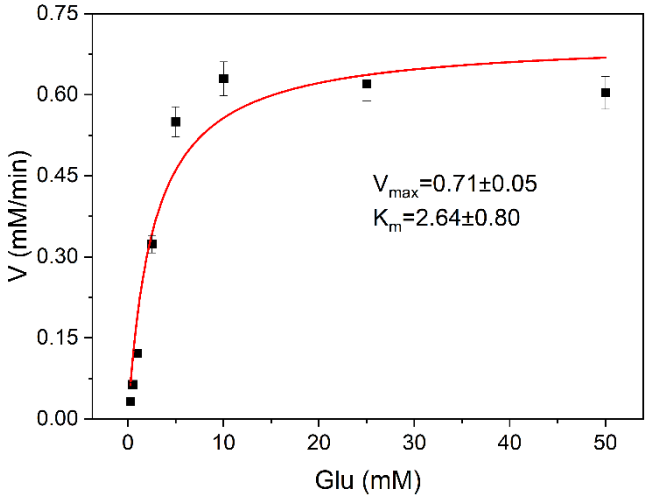

Fig. S7 Michaelis-Menten enzyme kinetic plot of GAD (V vs substrate concentration) Conditions: 0.1 M PBS (pH 5.0), 2 mL system containing 0.5–50 mM Glu, 0.1 mM PLP, and 0.95 μM GAD. The curve represents the model of best fit to the data, kinetic parameters V_m and K_m were derived from the fitting.

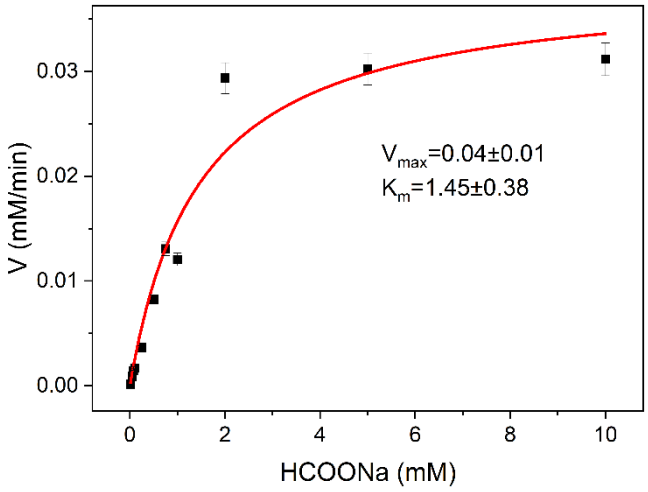

Fig. S8 Michaelis-Menten enzyme kinetic plot of FDH (V vs substrate concentration) Conditions: 0.001–1 mM HCOONa, 0.1 mM NAD^+ and 1.1 μM FDH in 0.1 M PBS (pH 7.0). The curve represents the model of best fit to the data, kinetic parameters V_m and K_m were derived from the fitting.

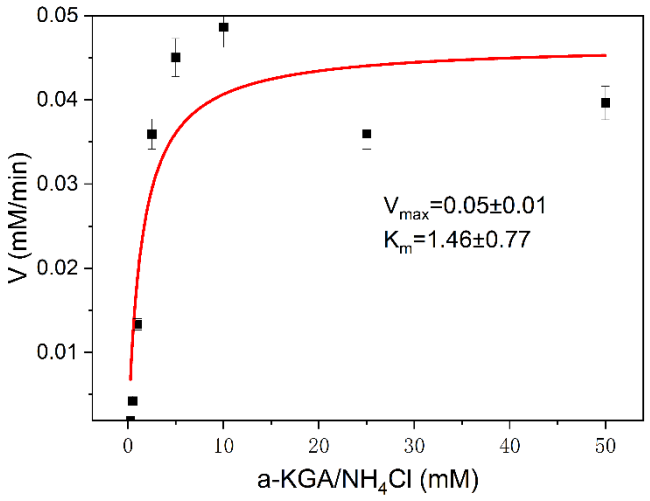

Fig. S9 Michaelis-Menten enzyme kinetic plot of GluDH (V vs substrate concentration). Conditions: 0.1 M PBS (pH 7.0), with 0.25–50 mM α-KGA/NH_4Cl, 0.1 mM NADH, and 0.9 μM GluDH. The curve represents the model of best fit to the data, kinetic parameters V_m and K_m were derived from the fitting.

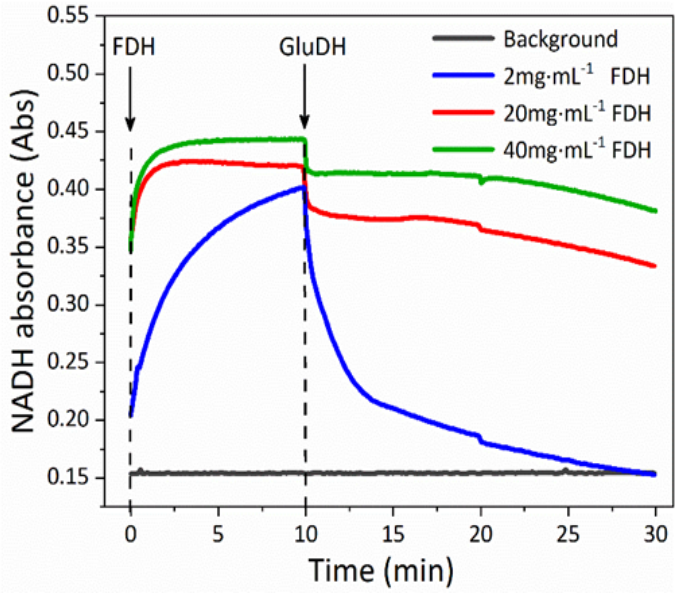

Fig. S10 UV-Vis spectra monitoring the cascade reaction between FDH and GluDH. Reaction conditions: 0.1 M PBS (pH 7.0), 0.1 mM NAD^+, 5 mM HCOONa, 5 mM α-KGA/NH_4Cl. Detection wavelength: 340 nm.

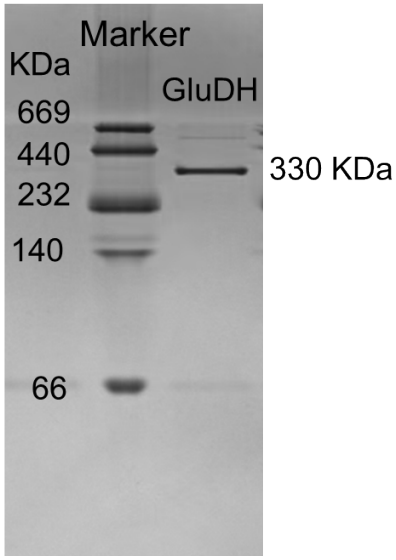

Fig. S11 Native page verification of the GluDH hexameric state.

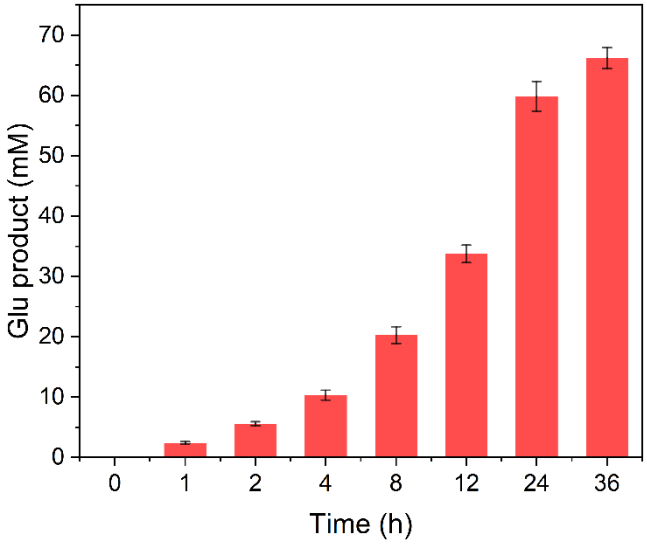

Fig. S12 Glu production by the cascade catalysis of FDH and GluDH over 36 hours. Reaction conditions: 5 mL of 0.1 M PBS (pH 7.0), 0.1 mM NAD^+, 100 mM HCOONa, 100 mM α-KGA/NH_4Cl, 20 μM FDH, 1 μM GluDH.

Table S1. Comparison of GABA synthesis methods

Method	**Solvent**	**Yield**	**E-factor (g_{waste} / $g_{product}$)**	**References**
Chemical synthesis	Hexafluoroisopropanol	92%	>600	[1]
Fermentation	Water / Acetonitrile	25.61 g/L	~2.4	[2]
Enzymatic reaction	Choline chloride / Glycerol	78.3%	~2.15	[3]
Chemoenzymatic cascade Synthesis	Water / Ethanol	70%	>1200	[4]
Multi-enzymatic cascade synthesis	**Water**	**93.6%**	**2.00**	**This work**

References

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